

THE CLAIMS DEFINING THE INVENTION ARE AS FOLLOWS

- 1 A method for detecting methylated nucleic acids comprising the steps of:
(i) contacting a nucleic acid sample suspected of containing methylated nucleotides with an oligonucleotide sequence under suitable conditions for nucleic acid hybridization, said oligonucleotide sequence characterised in that,
(a) it comprises a first stem labeled with a fluorophore moiety, a loop sequence having a region of nucleotides complementary to at least a region of the nucleic acid sample, which region is susceptible to methylation, and a second stem labeled with a quencher moiety that is capable of quenching the fluorophore moiety when in spatial proximity to the fluorophore moiety; and
(b) the nucleotides forming the first stem are capable of moving into spatial proximity with the nucleotides forming the second stem when the probe is dissociated from the nucleic acid sample;
(ii) altering the hybridization conditions such that the oligonucleotide probe dissociates from unmethylated DNA but remains hybridized to methylated DNA; and
(iii) measuring the change in fluorescence
- 2 A method according to claim 1 wherein when the labeled oligonucleotide sequences dissociate from the target nucleic acid sample according to step (ii) the first and second stem hybridise together causing quenching of the fluorophore moiety.
- 3 A method according to claim 1 wherein the loop sequence contains at least about 10 nucleotides.
- 4 A method according to claim 1 wherein the loop sequence contains at least about up to 35 nucleotides.
- 5 A method according to claim 1 wherein the loop sequence contains at least about 25 nucleotides.

- 6 A method according to claim 1 wherein the loop sequence contains at least about from 15-20 nucleotides.
- 7 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 5 8 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a Myt-3 nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 34 10 A method according to claim 8 wherein the labelled oligonucleotide sequence is complementary to at least one of the sequences selected from the group consisting of:
- (i) 5' GCG GCG ACT CCG ACG CGT CCA GCC CGC GCT CC 3'
(ii) 5' TTA TAC CGC AGG CGG GCG AGC CGC GGG CGC TCG CT 3'
(iii) 5' CCG AGA GCC CTG CGG GGC CGC CCC TCC TGC TGG CG 3'
- 15 10 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a glutathione-S-transferase-II(pi) nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 5 20 11 A method according to claim 10 wherein the labelled oligonucleotide sequence is complementary to at least one of the sequences selected from the group consisting of:
- i) 5' CTC CAG CGA AGG CCT CGC GGC CTC CGA GCC TTA TAA G 3'
ii) 5' GGG GAC GCG GGC CGC GCG TAC TCA CTG GTG GCG A 3'
- 12 A method according to claim 1 wherein when the loop sequence is complementary to a portion of a calcitonin nucleic acid sequence that undergoes methylation when a cell transforms from a normal state to a cancerous state.
- 25 13 A method according to claim 1 wherein the method is used to detect abnormally methylated gene sequences in prostate cancer tissues.

- 14 A method according to claim 1 wherein the hybridization condition that is altered during the hybridization reaction is the temperature of the hybridization reaction.
- 15 A method according to claim 1 wherein the stem sequences do not hybridise to the target gene and are of a sufficiently short length to avoid non-specific binding between the stem and any other nucleic acid sequence in the reaction mixture.
- 16 A method according to claim 1 wherein the stem sequences are at least about 4 to 8 nucleotides in length.
- 10 17 A method according to claim 1 wherein at least a cytosine in at least one of the stem sequences contains a methylated cytosine residue.
- 18 A kit comprising a labeled oligonucleotide sequence as described herein, which is adapted to distinguish methylated and non-methylated nucleic acid sequences when used in the method according to claim 1.
- 15 19 A method according to claim 1 substantially as herein before described.

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